# Anatomical Studies of the Neotropical Cyatheaceae. I. Alsophila and Nephelea

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The taxonomic treatment of the Cyatheaceae has undergone numerous revisions as summarized in previous studies (Tryon, 1970; Gastony, 1973; Lucansky, 1974). Earlier research has shown the taxonomic importance of anatomy and morphology to the study of this group of ferns (Holttum & Sen, 1961; Sen, 1964; Lucansky, 1974; Lucansky & White, 1974). Yet, despite this renewal of interest in the tree ferns, comparative anatomical data are almost totally lacking for the neotropical species.

Tryon's (1970) revised classification of the Cyatheaceae recognizes three basic evolutionary lines among the squamate genera, based upon petiole scale characters. The genera *Alsophila* and *Nephelea*, with structurally marginate petiole scales having dark apical setae, constitute one evolutionary line. Similarities in the sporogenetic pattern, spore morphology, basal pinna structure, and potential for squaminate spine development also demonstrate a close phyletic relationship between these two genera (Gastony, 1973, p. 83).

An attempt is made to determine whether the proposed phyletic relationship between *Alsophila* and *Nephelea* is supported by anatomical data. Our knowledge of anatomical data for the New World tree ferns is also increased.

The following species were used in this study: *Alsophila salvinii* Hook., *A. engelii* Tryon, *Nephelea erinacea* (Karst.) Tryon var. *erinacea,* and *N. polystichoides* (Christ) Tryon. Voucher specimens are on file in the herbarium of Duke University. Developing shoot tips were collected in the moist, tropical, mountainous regions of Costa Rica and Venezuela. The plant materials were killed and fixed in formalin-acetic acid-alcohol (FAA) and sectioned on a "macrotome" (Lucansky, 1976b). The sections (slices) were partitioned into manageable sizes, dehydrated in a tertiary-butyl alcohol series, and embedded in paraffin (Johansen, 1940). Sections (8 $\mu$m) were made and stained with safranin-fast green. Parts of stained sections were photographed with a 35 mm Zeiss C35 camera, whereas entire sections (slices) were photographed with a 35 mm single-lens reflex camera.

## RESULTS AND DISCUSSION

Based on habit, stem and petiole indument, stelar pattern, and nodal anatomy of mature specimens, *Alsophila* and *Nephelea* show striking similarities and a close phyletic relationship (Lucansky, 1974; Lucansky & White, 1974). Members of both genera are arborescent, with the upright habit being the derived condition in the Cyatheaceae (Bower, 1912, p. 293). Both genera are characterized by petiole scales that are differentiated into body and marginal cells and possess an apical seta. The cellular differentiation of the petiole margin in some species of *Alsophila* is similar to that of *Nephelea*. Certain species of both genera may bear setae on

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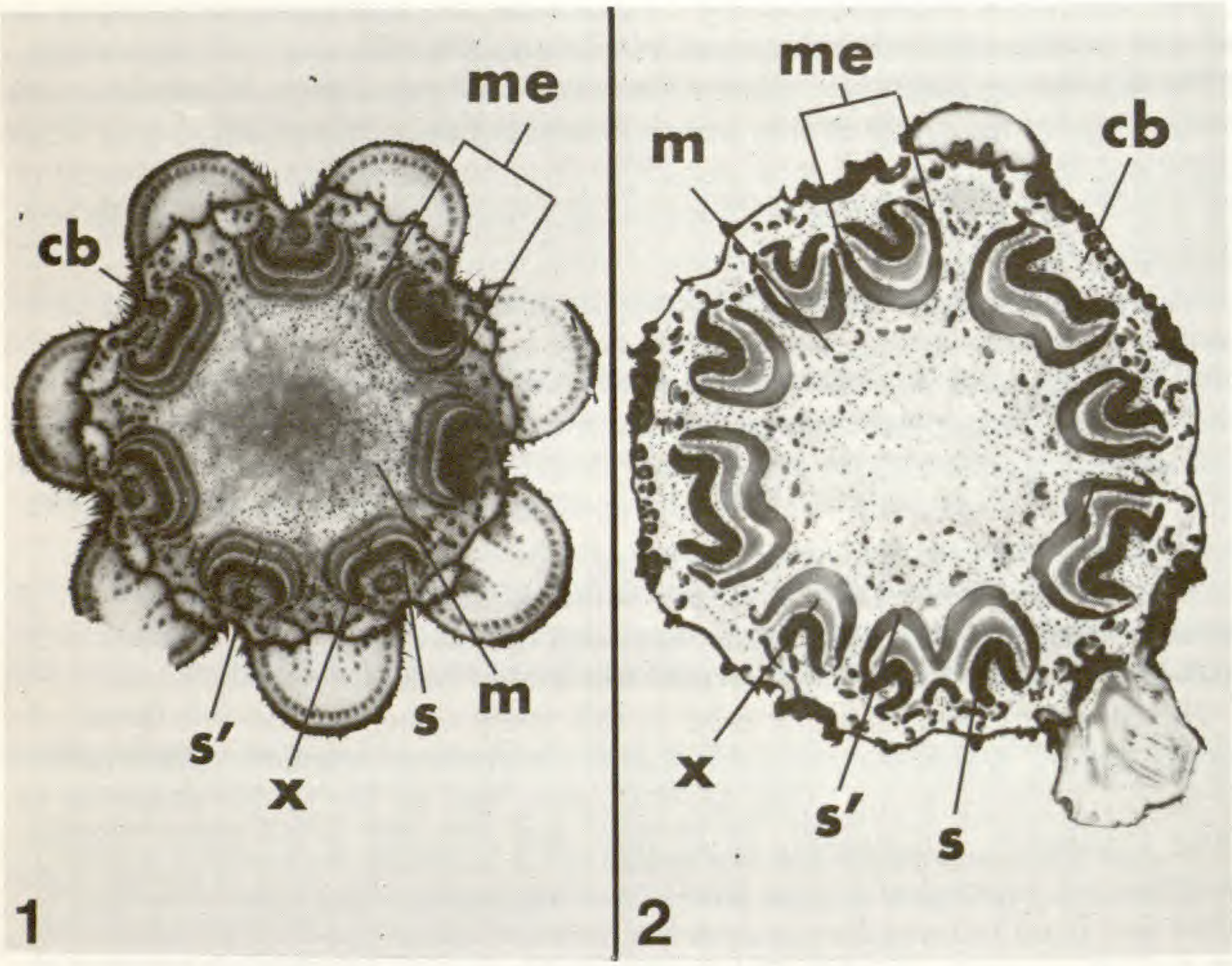
the margin or body of the scale (Tryon, 1970). *Alsophila* petioles typically lack spines, but if present they are corticinate, whereas *Nephelea* petioles have black, squaminate spines. Yet, squaminate spine development similar to that in *Nephelea* has been observed in *A. auriculata* (Tard.) Tryon (Tryon, 1970, p. 26).




Transections of tree-fern stems (dictyosteles). FIG. 1. *Nephelea erinacea* var. *erinacea*, × 0.7. FIG. 2. *Alsophila engelii*, × 1.2. cb = cortical bundle, m = medullary bundle, me = meristele, s = external stelar sheath, s′ = internal stelar sheath, x = xylem.

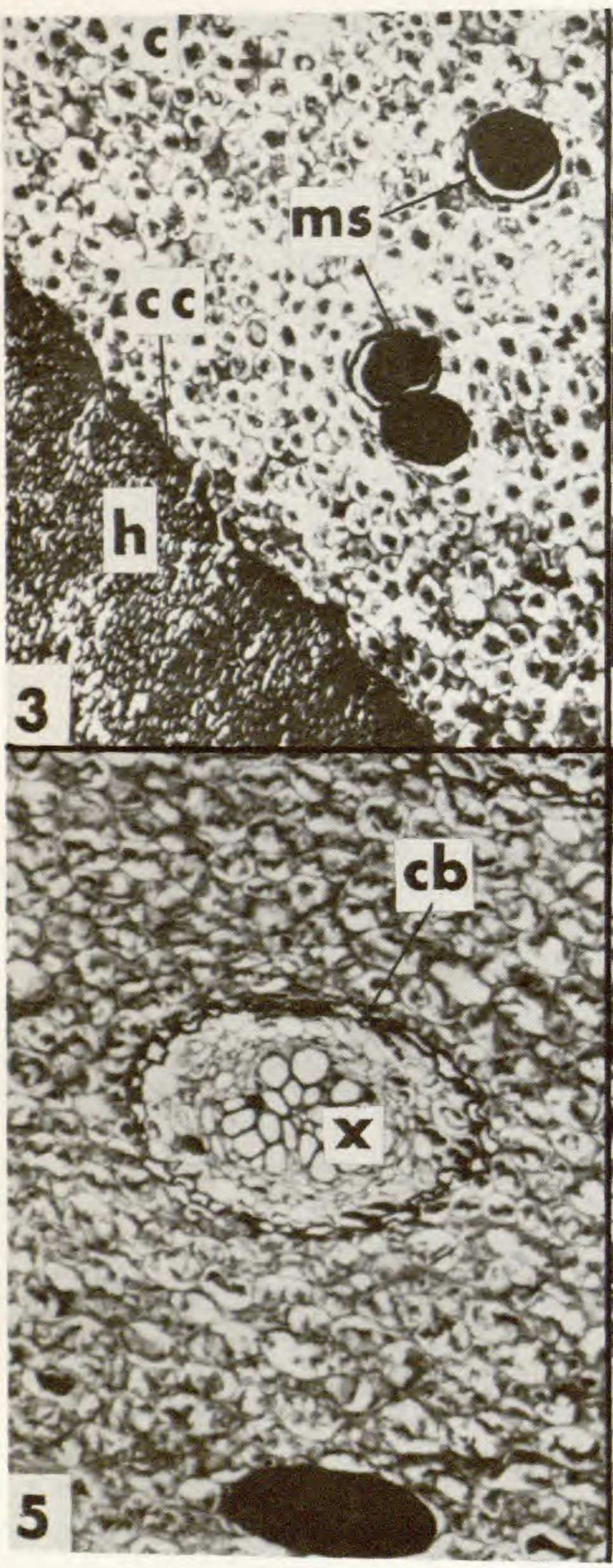
The stelar pattern in the mature stem is similar in all squamate genera, including *Alsophila* and *Nephelea* (Lucansky, 1974). In all species in this study the stelar pattern is a dictyostele composed of individual meristeles each surrounded by sclerenchymatous tissue (*Figs. 1* and *2*). The dictyostelic pattern is the result of the overlapping and lengthening of leaf gaps.

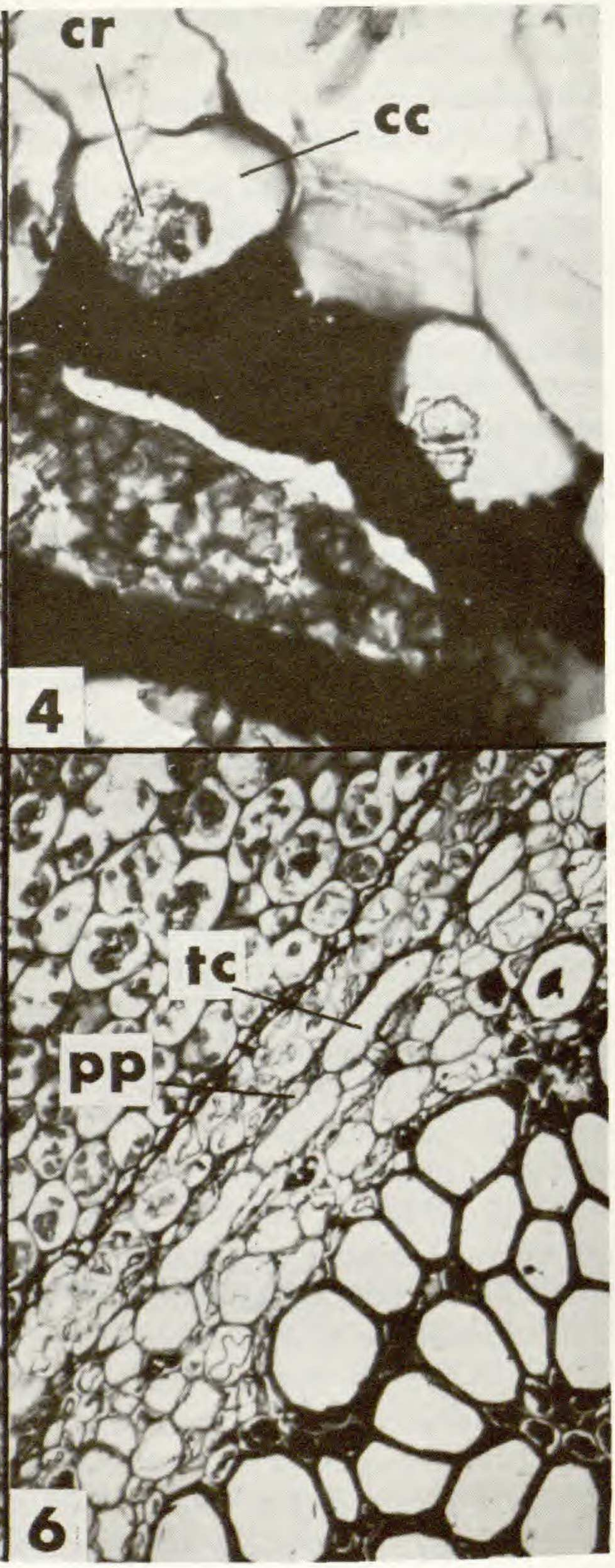
Stem transections of *Alsophila* and *Nephelea* reveal similar anatomical features. A single-layered epidermis composed of either elongate or irregularly-shaped cells is typically sloughed off in mature sporophytes. In *Culcita* and *Cystodium*, the outer walls of these cells may be thickened (Sen & Mittra, 1966) or cutinized (Sen, 1968). The outermost layer of the stem in *Alsophila* and *Nephelea* is a hypodermis composed of two zones of variable thickness, as previously reported (Ogura, 1927, pp. 174, 211; Ogura, 1938, p. 354; Sen, 1964). The outer zone is composed of thick-walled, irregularly-shaped parenchyma cells that typically are partially sloughed off. Sen (1968) reported that in *Culcita macrocarpa*

Presl these cells are formed by secondary sclerosis of the walls with the retention of the nucleus. The inner zone consists of sclerenchyma fibers (with lateral wall pitting) that are more isodiametric, smaller in diameter, and possess thinner walls than the parenchyma cells. Infrequently a middle zone composed of transitional parenchyma-sclerenchyma cells occurs in *N. polystichoides*, or the hypodermis is composed solely of sclerenchymatous fibers, as in *A. salvinii*. Ogura (1938, p. 354) also reported that the hypodermis may be composed entirely of sclerenchyma fibers. In all species in this study, the cortex is composed of large, irregularly shaped parenchyma cells with numerous starch grains, although Sen (1964) reported that a band of sclerenchyma tissue may occur between cortical layers of parenchyma in *Dicksonia* and *Culcita*. In the present study, mucilage-sac cells are randomly distributed in the cortex, either singly or in groups of 2 or 3 (*Fig. 3*), and form an articulated laticifer system. Schütze (1906) called these idioblasts excretion containers, rather than secretion cells, and reported that they contain fatty acids or tannin, whereas Ogura (1938, p. 356) found that they contained slime. Although no mucilage-sac cells are found in *Culcita* (Christensen, 1938) or *Cibotium barometz* (L.) J. Smith (Ogura, 1927, p. 277), such idioblasts have been reported for *Dicksonia squarrosa* (Forst.) Swartz (Williams, 1925). Previous workers have found cubical cells in the cortex of *Cyathea* and *Culcita* (Sen, 1964) and irregular patches of sclerenchyma tissue and intercellular spaces in the cortical zone of *Cystodium sorbifolium* J. Smith (Sen & Mittra, 1966).

A distinctive layer occurs between the hypodermis (sclerenchyma fibers) and the cortex (parenchyma cells). The cells that comprise this layer are greatly thickened on three walls (the wall proximal to cells of cortex remains thin-walled), and each cell contains a single, large irregular crystal (*Fig. 4*). These cells have been designated "cubical cells" by earlier workers (Ogura, 1927, p. 176, 1938, p. 354; Holttum & Sen, 1961; Sen, 1964). However, occasionally they do not possess a distinctive shape. Hence, the designation "cubical" is not warranted in all species. In transection the greatly thickened walls typically mask all features of this layer (*Fig. 3*), whereas in longitudinal section the isodiametric shape (if present) and large crystal are readily visible (*Fig. 4*). These cells are thick-walled parenchyma cells, based upon their living protoplast, wall morphology, position, and resemblance to parenchyma cells in younger stems. Sen (1964) felt that the cubical cells were not sclerenchyma cells, based upon their position, rate of cell division, and cellular inclusions, whereas Ogura (1938) reported that they were sclerenchyma cells. The crystalloid mass in each cubical cell is insoluble in $H_2SO_4$ (Holttum & Sen, 1961) and is thought to be composed of silica (Sen, 1968). In this study, the cubical cells form a continuous, distinctive layer, which infrequently is two cells thick in *N. polystichoides*. Numerous cubical cells are randomly distributed in the cortex of *Dicksonia squarrosa* and *Thyrsopteris* (Sen, 1964), whereas solitary cubical cells occur in *Lophosoria* (Holttum & Sen, 1961).

In *N. polystichoides*, *N. erinacea* var. *erinacea*, and *A. engelii*, vascular bundles occur in the cortical zone (Lucansky, 1974); such bundles are found only in certain members of the Cyatheaceae. These vascular bundles are surrounded by a distinct endodermis filled with tanniferous substances (*Fig. 5*). A pericycle of 1-3







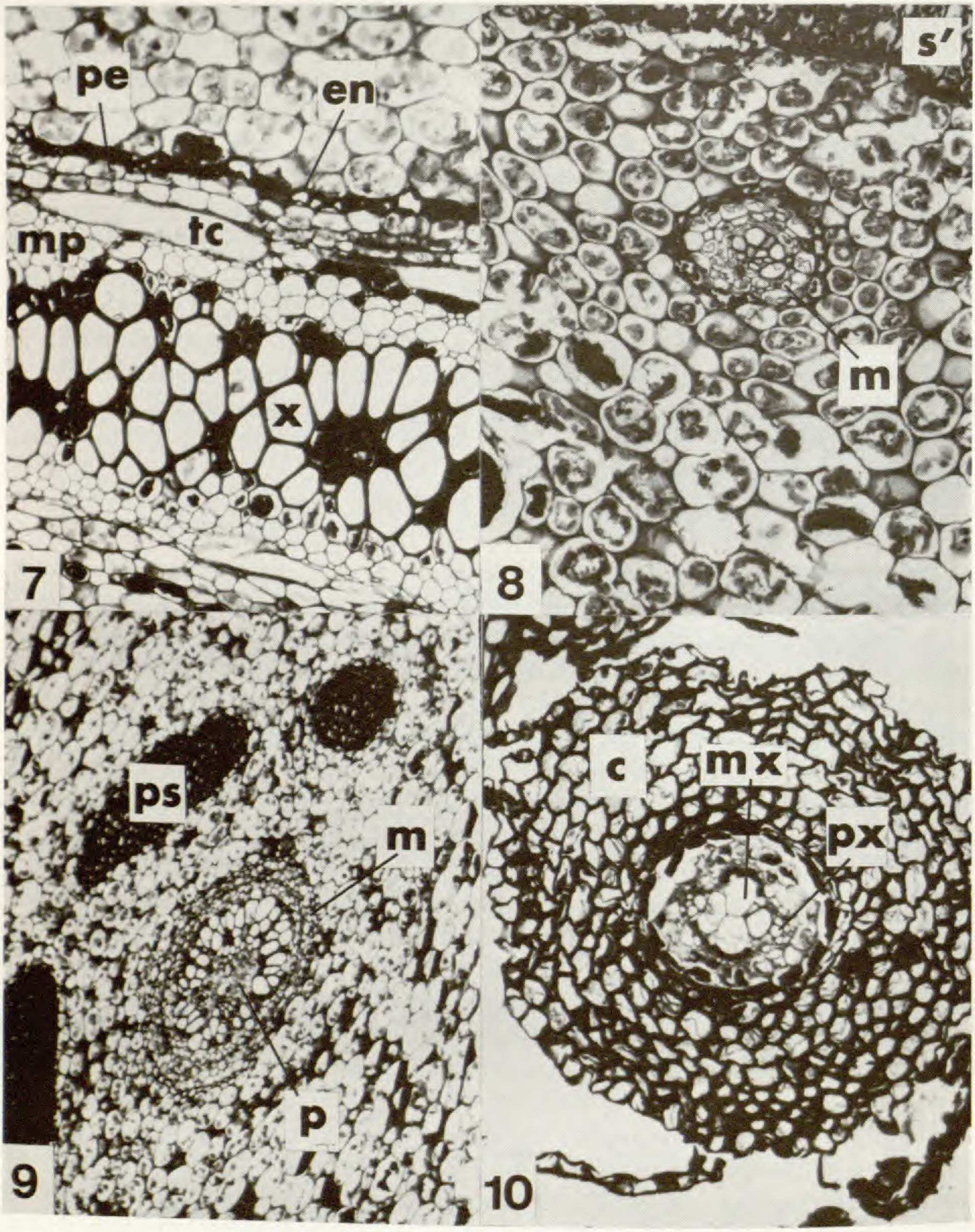



Anatomical details of tree-fern stems. FIG. 3. Mucilage-sac cells (laticifers) in cortical zone of *Nephelea polystichoides*, × 117. FIG. 4. Cubical cells with crystals in *Alsophila salvinii* forming boundary between hypodermis and cortex, × 31. FIG. 5. Small cortical bundle in *N. polystichoides*, × 128. FIG. 6. Tangential cells in meristele of *N. erinacea* var. *erinacea*, × 109. c = cortex, cb = cortical bundle, cc = cubical cells, cr = crystal, h = hypodermis, ms = mucilage-sac cell, pp = protophloem, tc = tangential cell, x = xylem.

layers of parenchyma cells encircles the primary phloem, which is composed of both sieve cells and phloem parenchyma. In all species studied, no tangential cells are found in the cortical bundles, regardless of their size. Depending upon the size of the cortical bundles, a parenchymatous pith may be found in the center of the primary xylem. Small bundles are protostelic (*Fig. 5*), whereas larger cortical bundles possess a parenchymatous pith. Ogura (1938, p. 362) reported that large bundles may have a cavity filled with tyloses. The primary xylem typically is composed of scalariform tracheids, with xylem parenchyma interspersed among these xylary elements in the larger bundles. Xylem maturation is mesarch. The smaller cortical bundles typically lack a sheath composed of sclerenchyma fibers, whereas the larger bundles may possess a partial sheath. In contrast, an earlier study indicated that those cortical bundles located along the external stelar sheath usually lack a sheath, and infrequently may be completely sheathed (Lucansky, 1974). The origin and fate of the cortical bundles is discussed in this earlier paper (Lucansky, 1974).

Transections of the stem show 3-10 meristeles, depending upon the species (*Figs. 1* and *2*). Each meristele is surrounded by an external and an internal stelar sheath composed of sclerenchyma fibers with conspicuous lateral wall pitting. The presence of sclerenchymatous tissue around the individual meristeles is a characteristic feature of the Cyatheaceae. The fibers of the stelar sheaths are typically longer and larger in diameter than those of the hypodermis. Both stelar sheaths are delimited externally and internally by a single layer of cubical cells, although the crystalloid mass is frequently lacking in these cells. Typically the external stelar sheath is more heavily lignified and thicker-walled than the internal stelar sheath, and appears darker in color (*Figs. 1* and *2*). The stelar sheaths, together with the hypodermis, provide support for the stem and the large leaves (Schütze, 1906). Both stelar sheaths arise from localized areas of sclerenchyma cells that undergo fusion to form a continuous sheath (Lucansky, 1976a). A parenchymatous zone that contains numerous mucilage-sac cells separates the stelar sheaths from the meristele and may function in the conduction and storage of carbohydrates (Schütze, 1906).

Each meristele is an amphicribral bundle and is delimited by a distinct endodermis filled with tanniferous substances. Distinct Casparian strips are lacking in the radial walls of these cells. A pericycle composed of 1-3 rows of parenchyma cells completely encircles the primary phloem. The latter tissue is composed of a single layer of protophloem (small, elongate cells), a distinctive layer of tangential cells, and several layers of metaphloem composed of sieve cells and phloem parenchyma (*Figs. 6* and *7*). Although Ogura (1927, p. 176, 1938, p. 361) reported that the protophloem was usually compressed, thick-walled, and swollen, no evidence of mechanical stress on these cells is found in this study (*Fig. 6*). Frequently the protophloem and metaphloem are indistinguishable in all species studied, or the former tissue may be lacking. Although earlier workers indicated that the primary phloem is composed of distinct layers (rows) of phloem parenchyma and sieve cells (Schütze, 1906, p. 353; Ogura, 1927, p. 177), these two cell types are randomly interspersed in the species in the present study. The sieve




Anatomical details of tree-fern stems. FIG. 7. Meristele of *A. salvinii* showing tangential cells, × 171. FIG. 8. Small medullary bundle near internal stelar sheath in *A. engelii*, × 110. FIG. 9. Large medullary bundle with partial sheath and pith in *N. erinacea* var. *erinacea*, × 105. FIG. 10. Transection of root of *N. erinacea* var. *erinacea*, × 104. c = cortex, en = endodermis, m = medullary bundle, mp = metaphloem, mx = metaxylem, p = pith, pe = pericycle, ps = partial sheath, px = protoxylem, s′ = internal stelar sheath, tc = tangential cell, x = xylem.

cells possess scalariform sieve plates and sieve areas on their lateral walls. Sen (1964) found mucilage-sac cells in the primary phloem, whereas in the present study these cells are simply phloem parenchyma filled with mucilage.

The tangential cells are large and elongate tangentially in transection (*Fig. 6*), and form a characteristic feature of the Cyatheaceae. These cells typically occur between the protophloem and metaphloem, but may represent the outermost layer of the primary phloem, if the former layer is lacking. They represent specialized sieve cells that are devoid of nuclei, possess sieve areas on their lateral walls, and accumulate callose (Sen, 1964), although they have been variously referred to as "false sieve tubes" (Schütze, 1906, p. 355) or mucilage cells (Ogura, 1927, p. 341). According to Ogura (1927, p. 342), these distinctive cells may be partially or entirely replaced in a given species by mucilage cells or longitudinally elongate cells (i.e., tangential cells are mucilage cells with a different orientation).

The primary xylem typically is composed of tracheids with scalariform end plates. The xylem parenchyma contains mucilage droplets interspersed among the tracheids. Each meristele is composed predominantly of metaxylem, with the protoxylem poles difficult to discern (*Fig. 7*). Earlier studies have reported that protoxylem is usually absent in the primary xylem of mature stems (Ogura, 1927; Sen, 1964), although spiral tracheids are noted infrequently in the present study. Xylem maturation is mesarch in all species studied.

The pith is composed of large, irregularly-shaped parenchyma cells that contain numerous starch grains. Mucilage-sac cells are randomly distributed in the pith, either singly or in groups of 2 or 3. A distinctive cubical layer occurs between the internal stelar sheath and the pith, although crystals typically are lacking in these cells.

In all species studied, medullary bundles are scattered randomly in the pith (*Figs. 1* and *2*), and represent a characteristic feature of the Cyatheaceae. The number of these bundles varies according to the size of the pith (Lucansky, 1974). These accessory bundles are identical in cellular composition to the cortical bundles (*Figs. 5* and *8*). Ogura (1927, 1938, p. 362) reported that the larger bundles may contain a central cavity with tyloses, although none are noted in the present study. In the species studied, small medullary bundles are protostelic, whereas larger bundles possess a parenchymatous pith (*Figs. 8* and *9*). Occasionally a very large bundle may possess several parenchymatous areas within the primary xylem. Tangential cells typically are lacking in medullary bundles, although the largest bundles may possess these distinctive cells. Ogura (1927) also reported the absence of tangential cells in the medullary bundles of certain species, whereas Schütze (1906, p. 365) found that these cells were the major component of the primary phloem of these bundles in *A. manniana* (Hooker) Tryon (as *Cyathea usambarensis* Hieron.). The primary xylem is composed primarily of scalariform tracheids, with no protoxylem visible. Xylem maturation is mesarch in these bundles (Lucansky, 1976a). A partial sheath composed of sclerenchyma fibers may or may not be found associated with each medullary bundle (*Fig. 9*). Normally only the vascular bundles located along the internal stelar sheath lack a sheath (*Fig. 8*), although Ogura (1938) reported that medullary bundles usually

lack such tissue. Larger bundles in the pith typically possess a more extensive partial sheath than that associated with cortical bundles. A thorough discussion of the origin and fate of medullary bundles is given in previous papers (Lucansky, 1974; Lucansky & White, 1974; Lucansky, 1976).

Transections of the adventitious roots show similar anatomical features in all species (*Fig. 10*). Typically the epidermis is sloughed off in mature roots, and the outer cortex, which is composed of thick-walled parenchyma cells, forms the outer boundary of the organ. These cortical cells are irregularly-shaped and partially sloughed off, and the inner cortex is composed of isodiametric sclerenchyma fibers and typically forms the bulk of the cortical zone. Ogura (1927, p. 340) indicated a similar arrangement for the cell layers that comprise the cortex, whereas other workers found the position of these cell layers reversed in the cortical zone (Schütze, 1906; Sen, 1968). The endodermis is a single layer composed of cells filled with tanniferous substances, but lacks distinct Casparian thickenings on the radial walls. A pericycle 1-3 cells thick and composed of large, irregularly-shaped parenchyma cells surrounds the vascular tissue. The primary phloem consists of sieve cells and phloem parenchyma. The xylem is typically diarch with exarch maturation (*Fig. 10*), although triarch and tetrarch xylem occasionally occur in larger roots. Longitudinal sections reveal primarily scalariform-pitted metaxylem, with some spiral and transitional (reticulate-scalariform) protoxylem.

Root traces originate either from the meristele or from the base of leaf traces and pass obliquely through the cortex. Ogura (1927) reported that the amount of xylem parenchyma increases and the number of tracheids diminishes during this passage.

Leaf traces arise at successive levels in the leaf gap and proceed to the petiole, where they form a much-dissected vascular pattern that is basically similar for all four species studied (Lucansky & White, 1974). The leaf traces vary in shape and are spherical-ellipsoidal to horseshoe-shaped, depending upon proximity to the petiole base. They are identical in cellular composition to the accessory bundles. The xylary elements are primarily metaxylem, with protoxylem located at the concavity of the vascular tissue in the horseshoe-shaped configuration.

The individual petiole strands are horseshoe-shaped and vary in number, depending upon the genus (Lucansky & White, 1974). The single-layered epidermis is sloughed off in mature plants, and a thick-walled parenchymatous zone represents the peripheral layer of the petiole. The ground tissue, composed of thin-walled parenchyma cells containing numerous mucilage droplets, comprises the bulk of the petiole. Cellular composition of the stele of each petiole strand (U- or V-shaped) is similar to that of a leaf trace, with protoxylem restricted to a median position on the concave side of the vascular tissue. Although previous workers reported that the protoxylem may partially disintegrate and form a cavity with tyloses (Schütze, 1906; Ogura, 1927, p. 343), none are found in the present study.

Based upon comparative anatomical data, *Alsophila* and *Nephelea* show striking similarities and represent closely-related genera. These data also support recent phyletic conclusions for these taxa (Tryon, 1970; Gastony, 1973, 1974).

## LITERATURE CITED

BOWER, F. O. 1912. Studies in the phylogeny of the Filicales, II. Lophosoria, and its relation to the Cyatheoideae and other ferns. Ann. Bot. **26:** 269-323.

CHRISTENSEN, C. 1938. Filicinae. *In* F. Verdoorn, (ed.). Manual of Pteridology. Martinus Nijhoff, The Hague.

GASTONY, G. J., 1973. A revision of the fern genus Nephelea. Contr. Gray Herb. **203:** 81-148.

———. 1974. Spore morphology in the Cyatheaceae. I. The perine and sporangial capacity: general considerations. Amer. J. Bot. **61:** 672-680.

HOLTTUM, R. E., and U. SEN. 1961. Morphology and classification of the tree ferns. Phytomorphology **11:** 406-420.

JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill, New York.

LUCANSKY, T. W. 1974. Comparative studies of the nodal and vascular anatomy in the neotropical Cyatheaceae. II. Squamate genera. Amer. J. Bot. **61:** 472-480.

———. 1976a. Comparative ontogenetic studies in young sporophytes of tree ferns. I. A primitive and an advanced taxon. Amer. J. Bot. **63:** 463-472.

———. 1976b. The macrotome: a new approach for the sectioning of large plant specimens. Stain Technol. **51:** 199-201.

———, and R. A. WHITE. 1974. Comparative studies of the nodal and vascular anatomy in the neotropical Cyatheaceae. III. Nodal and petiole patterns; summary and conclusions. Amer. J. Bot. **61:** 818-828.

OGURA, Y. 1927. Comparative anatomy of the Japanese Cyatheaceae. J. Fac. Imp. Univ. of Tokyo (Bot.) **1:** 41-350.

———. 1938. Handbuch der Pflanzenanatomie, Anatomie der Vegetationsorgane der Pteridophyten. Band VII, Teil 2. Gebrüder Borntrager, Berlin.

SCHÜTZE, W. 1906. Zur physiologischen Anatomie einiger tropischer Farne, besonders der Baumfarne. Beitr. Wiss. Bot. **5:** 329-376.

SEN, U. 1964. Importance of anatomy in the phylogeny of tree ferns and their allies. Bull. Bot. Soc. Bengal **18:** 26-34.

———. 1968. Anatomy of Culcita macrocarpa. Canad. J. Bot. **46:** 43-46.

———, and D. MITTRA. 1966. The anatomy of Cystodium. Amer. Fern. J. **56:** 97-101.

TRYON, R. 1970. The classification of the Cyatheaceae. Contr. Gray Herb. **200:** 1-53.

WILLIAMS, S. 1925. Some points on the anatomy of Dicksonia. Proc. Roy. Soc. Edinburg **45:** 286-296.